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Attorney's Docket No. 33339/206076 PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Laurent Benbadis *et al.*	Confirmation No.:	
Appl. No.:	09/700,687	Group Art Unit:	1651
Filed:	February 14, 2001	Examiner:	R. Davis
For:	MUTANT LACTOBACILLUS BULGARICUS STRAINS FREE FROM BETA-GALACTOSIDASE ACTIVITY		

Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. 1.132

Sir:

I, J. Mengaud, do hereby declare and say as follows:

1.

CURRICULUM VITAE .

Jérôme MENGAUD
Birth date : September 4th 1965
French Nationality

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Current professional activity (since 2001) : Research group leader – Culture and Yeast

Previous professional activity :

1996 - 2001 : Research engineer in the Starter an Yeast group of the Danone Research center
1993 - 1996 : Research assistant at the Pasteur Institute in Paris, Bacteria-Host interaction unit (P. Cossart)
1992 - 1993 : Post-doctoral position at the University of California Los Angeles (M. Horwitz's laboratory)

Education :
- pHD thesis in Microbiology prepared at the « Institut National d'Agronomie – Paris-Grignon » and the Pasteur Institute - Paris (December 1990) Under the Scientific supervision of P. Cossart :
Genetic analysis of *L. monocytogenes* virulence factors: role of listeriolysin O and other virulence factors.

- Engineer diploma of the « Institut Agronomique Paris-Grignon » (1987)

- DEA of microbiology of the university Paris VII (1987)

2. Since 1996, I have been working under the supervision of L. Benbadis, F. Gendre and then S. Veillet.

3. I conducted and supervised experiments to compare the acidification properties exhibited by 2 independent *L. bulgaricus* lacZ non-sense mutants each derived from a different wild type *L. bulgaricus* parent strain.

4. The experiments were conducted in the following manner.

5. In order to compare the acidification properties of the two lacZ non-sense mutants, the following strains of *L. bulgaricus* were used:

a) Non-sense mutant strain 1-1968 disclosed in the instant application, which is derived from wild type strain LbS and has a mutation in the *LacS* gene resulting in an Asn residue at position 122 of SEQ ID NO: 2 (in place of a Lys residue in the parent strain LbS), and a non-sense mutation in the *lacZ* gene introducing a stop codon resulting in a β-galactosidase truncated after position 880, *i.e*, downstream the active sites.

b) Non-sense mutant strain DN-100 562, which is derived from wild type strain DN-100 339. Strain DN-100 562 has no mutation in the *LacS* gene, and a non-sense mutation in the *lacZ* gene introducing a stop codon resulting in a β-galactosidase truncated after position 43, *i.e.*, upstream the active sites.

6. These mutant strains are schematized in Figure 1.

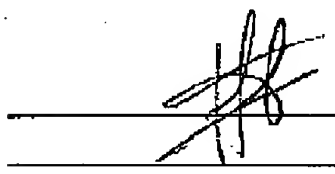
The parent strains LbS and DN-100 339 are independent and different. By way of example, strain DN-100 339 has a ropy phenotype, which is not the case for strain LbS.

7. For each strain, a first subculture (inoculated with 1% of an overnight culture) was prepared at 42°C in sterilized reconstituted skim milk complemented with 2g/l of yeast extract and 20g/l of glucose and used to prepare a second subculture under the same experimental conditions (inoculation at 1%). Reconstituted skim milk (12% w/vol) complemented with 0.5 g/l, 1g/l or 5 g/l of glucose was heated 30 min at 95°C, inoculated with 1% of this second subculture, and the kinetics of acidification determined by measuring the pH of the resulting solution every minute at 42°C using a cinac apparatus.

8. As the acidification data of Figure 2 show, strain I-1968 and strain DN-100 562, having both a non-sense mutation in the *lacZ* gene, present the same acidification properties, although they are derived from 2 independent and different wild-type strains, and although they differ in the localization of the non-sense mutation in the *lac Z* gene.

In both cases acidification takes place until all glucose is used and then stops. Addition of 5g/l of glucose in milk at the beginning allows in both cases to bring acidification to a lower pH than when only 1g/l or 0.5 g/l of glucose is added.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



September 9th 2004

J. Mengaud

Date